The Rhizosphere

The Ultrastructure of the Rhizosphere of *Trifolium subterraneum* L.

R.C. FOSTER and A.D. ROVIRA

A. Introduction

Early investigations of root fine structure using transmission electron microscopy (TEM) of whole mounted roots showed that the surface was coated with an amorphous granular mucilage (8) in which bacteria may be embedded. More recently the rhizosplane has been examined by scanning electron microscopy (SEM) (6) but neither of these techniques allows bacteria which have similar external morphology to be distinguished. Morphologically similar bacteria can be distinguished, however, in ultrathin sections of rhizospheres examined in the TEM (10, 11, 12, 18).

This investigation is part of a general study of the ultrastructure of the rhizospheres of agricultural or forest plants aimed at widening our understanding of rhizosphere ecology with particular reference to root pathogens. In this paper we describe the rhizosphere of Trifolium subterraneum L. and compare it with that of plants grown in the same soil (12).

B. Materials and Methods

Undisturbed cores each containing one plant of Trifolium subterraneum L. cv Bacchus Marsh were obtained as described previously (22).

C. Results

Figures 1 and 2 are generalised low-power views of rhizospheres of lateral roots of clover plants at the flowering stage. To the left in both figures are the outer cortical cells of the root (R), perhaps overlain by collapsed root cap remnants. The cells have been colonised by soil microorganisms and the cell walls have been lysed leaving the electron-dense lignified cell wall corners.

The rhizosphere organisms are embedded in a fibrous matrix which occupies most of the space between the root surface and the clay and organic soil particles (Figures 1 and 2). Many kinds of microorganism can be distinguished by their morphology (Figs. 3-6) and internal ultrastructure as well as by the structure of their capsule material (Figs. 7-9). The bacteria are closely packed, especially when close to the root surface (see Table 1), and many occur in distinctive small colonies about 5 to 10 µm in diameter so that only 4 or 5 cells appear in an ultrathin section. Some bacteria are surrounded by an extended capsule which can be

---

Fig. 1. General view of the rhizosphere. All the plates are transmission electron micrographs of transverse ultrathin sections of fine lateral roots of Trifolium subterraneum cv Bacchus Marsh. The colonies are labelled a-o in order of appearance on the plates. The bar indicates 1 µm unless otherwise stated on the figure. The following abbreviations are used: C, capsule material; M, soil minerals, mainly clay particles; PCWM, plant cell wall material; O, soil organic matter; R, clover root; V, virus ►

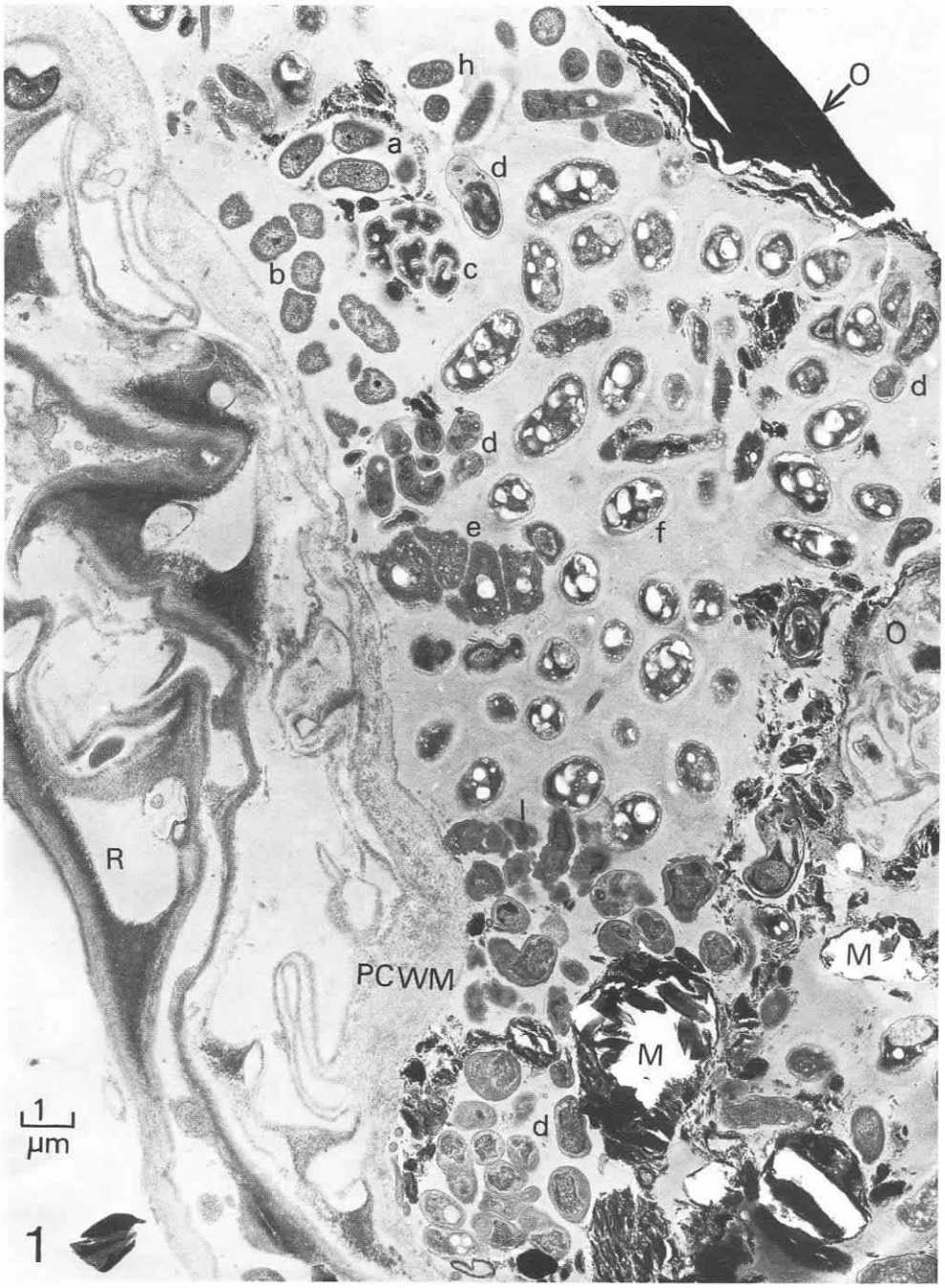


Fig. 1

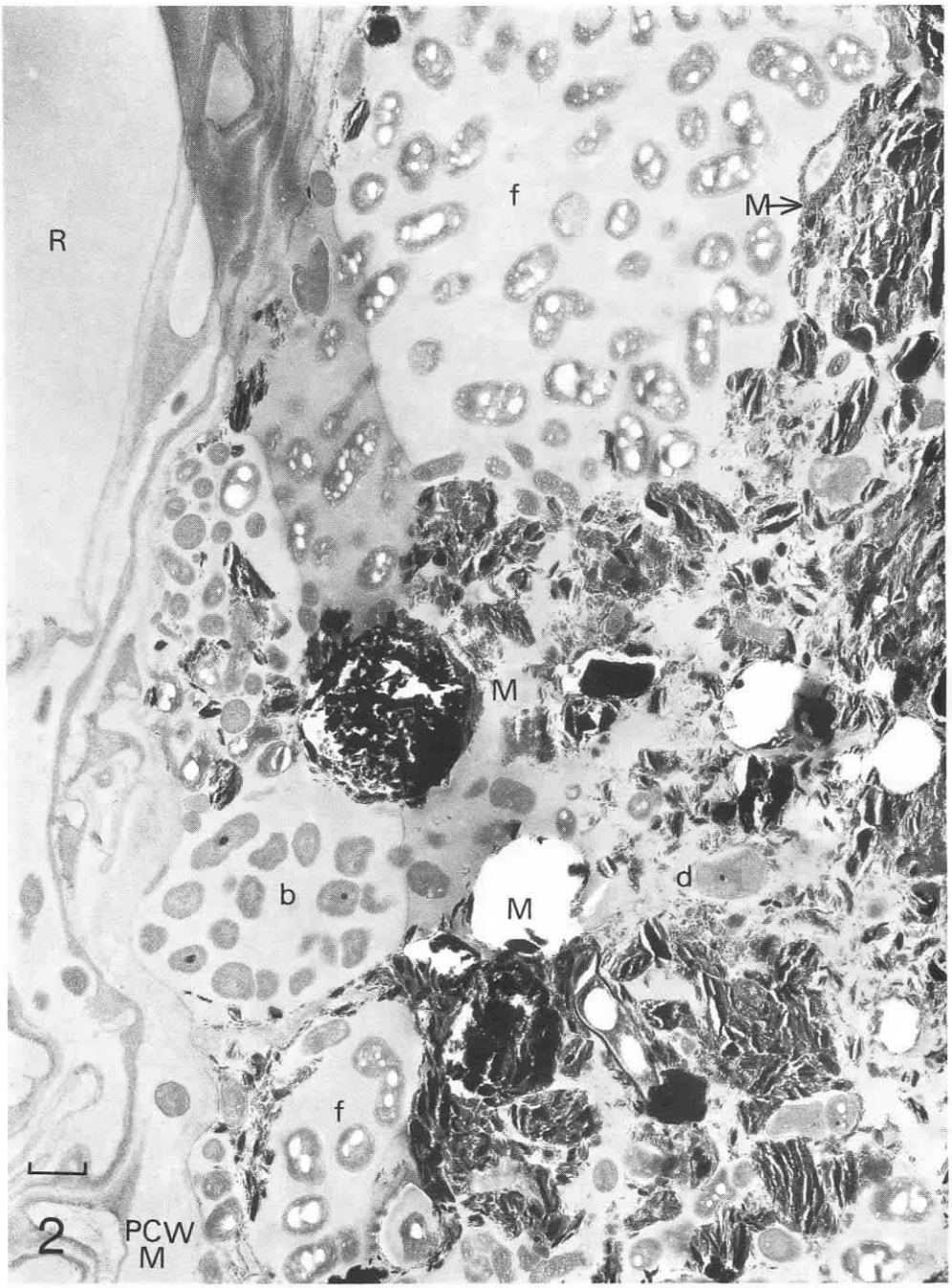
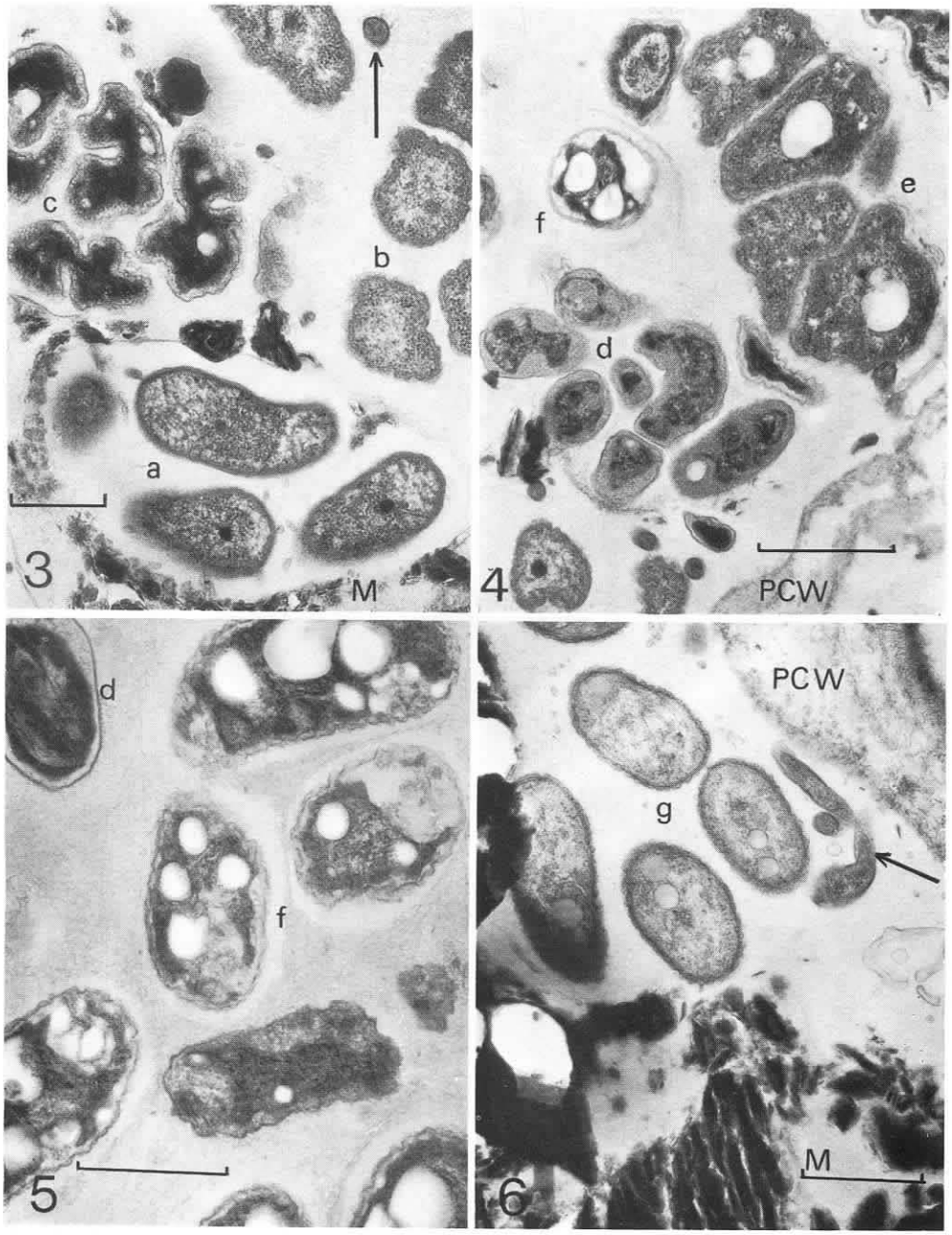
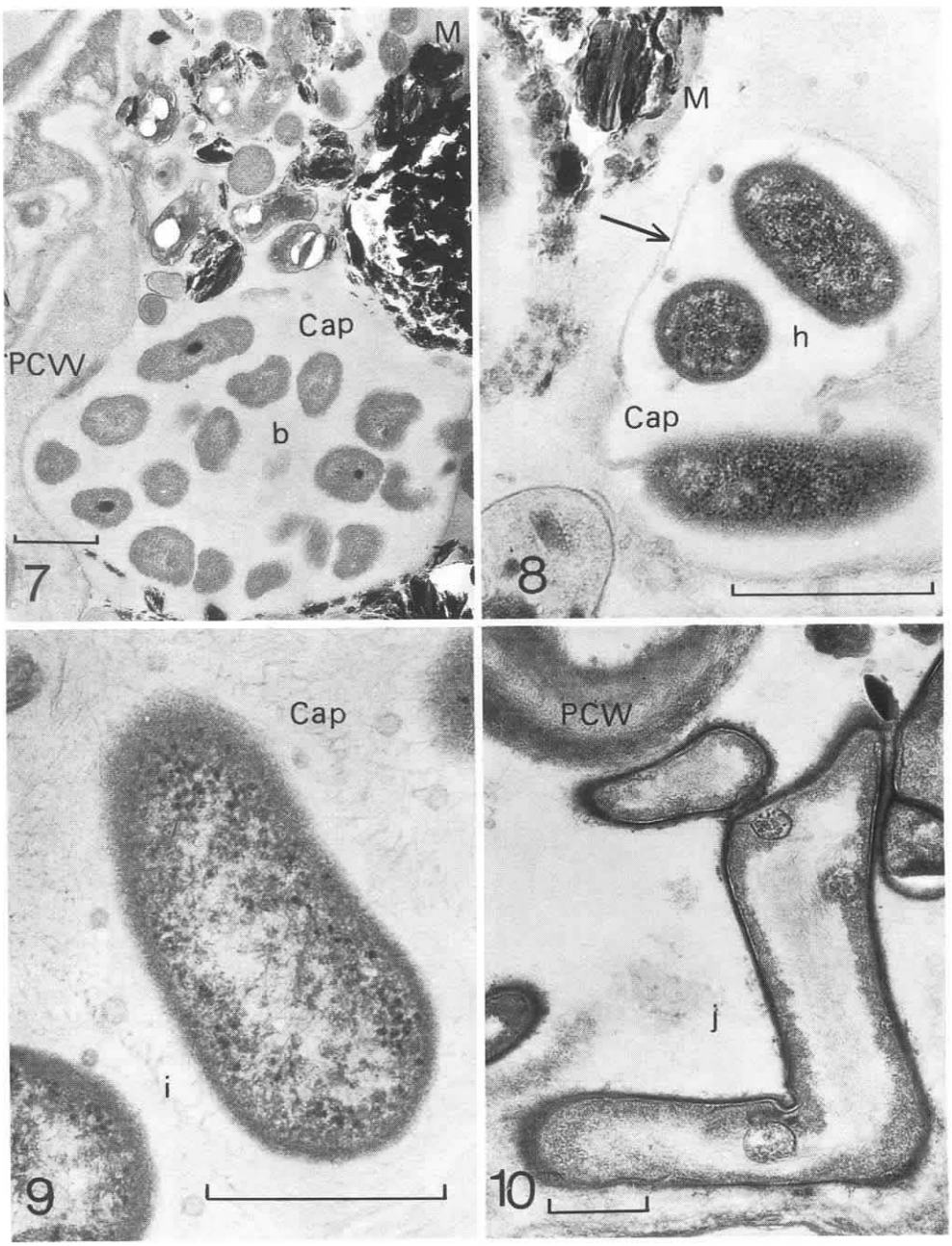


Fig. 2. General view of the rhizosphere (see legend to Fig. 1)



Figs. 3-6. Examples of rhizosphere organisms (see legend to Fig. 1)



Figs. 7-10. Examples of rhizosphere organisms (see legend to Fig. 1)

readily distinguished from the general rhizosphere matrix by its physical organisation and staining properties (Figs. 3-14).

After close examination of several low power electron micrographs we consider that there is evidence of zonation within the rhizosphere. Most of the distinctive small colonies appear to occur within 5 μm of the rhizoplane (Table 2) and the cells are more densely packed in this region (Table 1). Measurements of several hundred microorganisms from the rhizosphere indicated that the mean diameter was about 1 μm and we here define the rhizoplane as that extending from the root surface to 1 μm into the soil. It is convenient for purposes of comparison to define an inner rhizosphere 0 to 10 μm including the rhizoplane and an outer rhizosphere 10 - 20 μm from the root surface.

Some of the organisms encountered in clover rhizospheres are illustrated in detail in Figures 3-14 and they have been labelled alphabetically to correspond to the types in Table 2.

The most common organism profiles, are types 'd' and 'f'. Type 'f' has similar densities in the innermost zones but it comprises 70% of all the organisms in the outer rhizosphere compared with only 19% at the rhizoplane.

Table 1. Numbers of microorganisms in the rhizosphere

Zone	Distance from root in μm	Number of kinds of organism which can be distinguished cytologically	Estimated frequency cells per cc.
RHIZOPLANE	0-1	11	120×10^9
INNER	0-5	12	96×10^9
RHIZOSPHERE	5-10	5	41×10^9
OUTER	10-15	2	34×10^9
RHIZOSPHERE	15-20	2	13×10^9

Table 2. Populations of microorganisms in various zones of the rhizosphere

Zone	Distance from the root surface in μm	Numbers of individuals per cc of soil ($\times 10^{-9}$)										
		a*	b	c	d	e	f	g	h	l	m	ind**
RHIZOPLANE	0 - 1	-	13	-	27	3	23	10	-	13	3	3
INNER	0 - 5	2	5	2	21	3	24	2	-	8	2	7
RHIZOSPHERE	5 - 10	1	-	2	10	-	25	-	4	-	-	1
OUTER	10 - 15	-	-	-	9	-	23	-	-	-	-	2
RHIZOSPHERE	15 - 20	-	-	-	2	-	4	-	-	-	-	2

* type of organism profile, see figures and text

** type indistinguishable cytologically

In Figure 2 the outer regions of the rhizosphere have been included in the section and they contain clay minerals in higher concentration than in the inner rhizosphere, but bacteria can be found amongst the grains over 20 µm from the rhizoplane. In some cases the bacteria appear to be closely enclosed by the clay grains and it is possible that these are inside specialised microsites where bacterial growth may be inhibited by lack of space or nutrients. They may, however, be sites in which bacteria can survive changes in soil pH due to the buffering action of ions on the mineral surfaces and changes in soil moisture since water may be retained in the fine capillaries within the clay particles. They may also be protected from predators (21). Some microorganisms have bizarre shapes (Figure 3, colony 'c', Figures 11 to 18) but even where they have more usual shapes the organisms can often be distinguished by their characteristic ultrastructure.

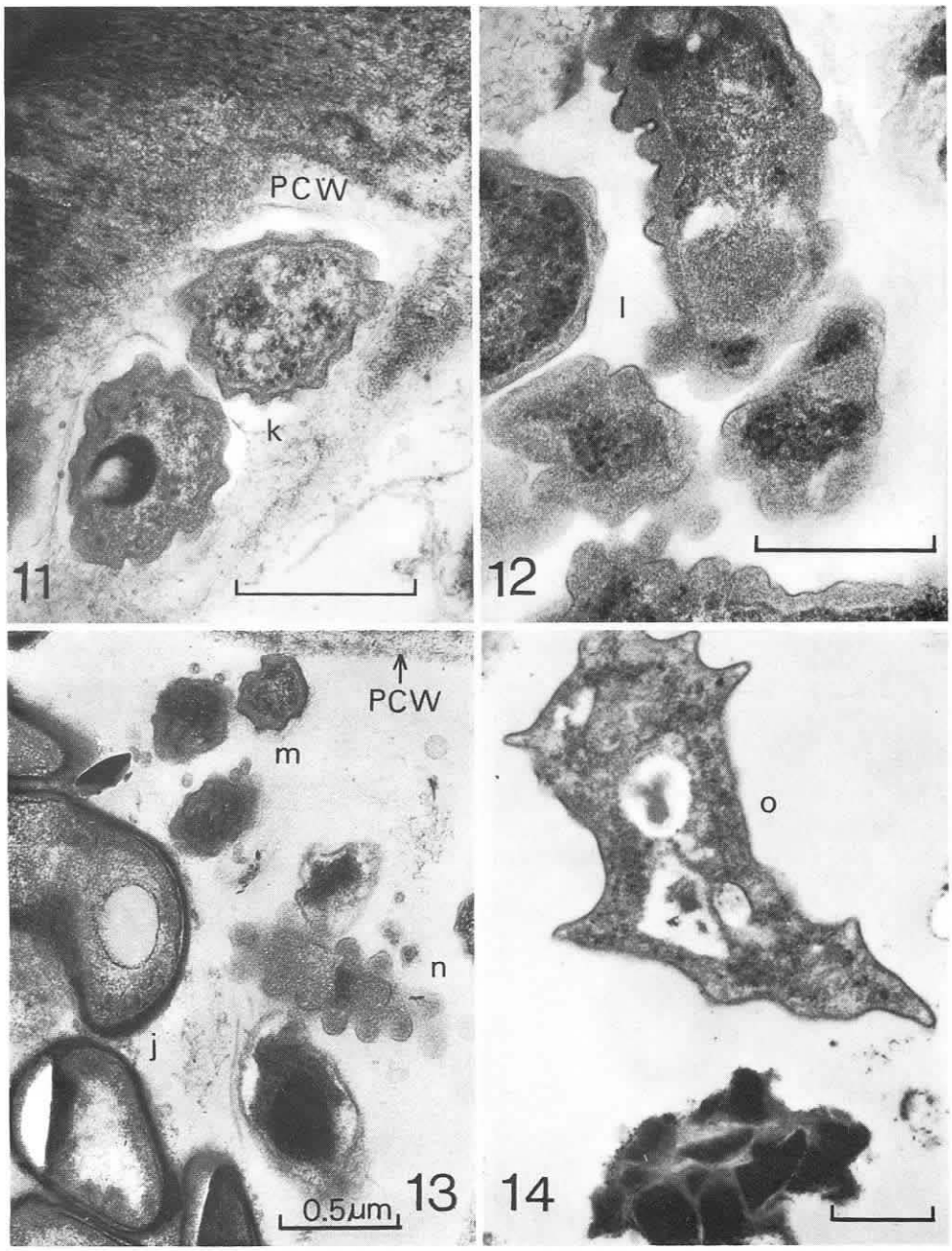
Some Trifolium rhizospheres contain actinomycete-like organisms often very close to the root ('j' Figure 10). These organisms have branching, filamentous cells with septae and prominent mesosomes. They have a Gram positive type of wall which encloses a granular, densely packed peripheral cytoplasm surrounding a fibrous nucleoid region. There is no extended capsule and the cells lie in the general rhizosphere matrix.

Figures 11-14 show some of the more uncommon lobed forms which, in our experience, are usually found only within the root or very near the rhizoplane. Similar forms have been seen by us in wheat and Pinus radiata rhizospheres, again always within a few microns of the host cell wall or in the crevices between host epidermal cells and they seem to be restricted to this specialized environment. Associated with these cells is an unusual helically lobed organism 'n' about 0.25 µm in diameter and 0.5 µm long, similar to an organism recently described by Old et al.(19) in rhizospheres of grasses from dune sands. Occasionally large lobed (protozoan ?) organisms are encountered, 4 or 5 µm long. Protozoa are common in soils and may be expected in areas with high bacterial populations such as the rhizosphere. Such unusual types of microorganisms are seldom isolated in this form from soils by the usual plating techniques, and it is possible that they are too firmly enclosed by the rhizosphere mucigel to be removed by washing, or that they have stringent nutrient requirements which cannot be met in the usual culture media.

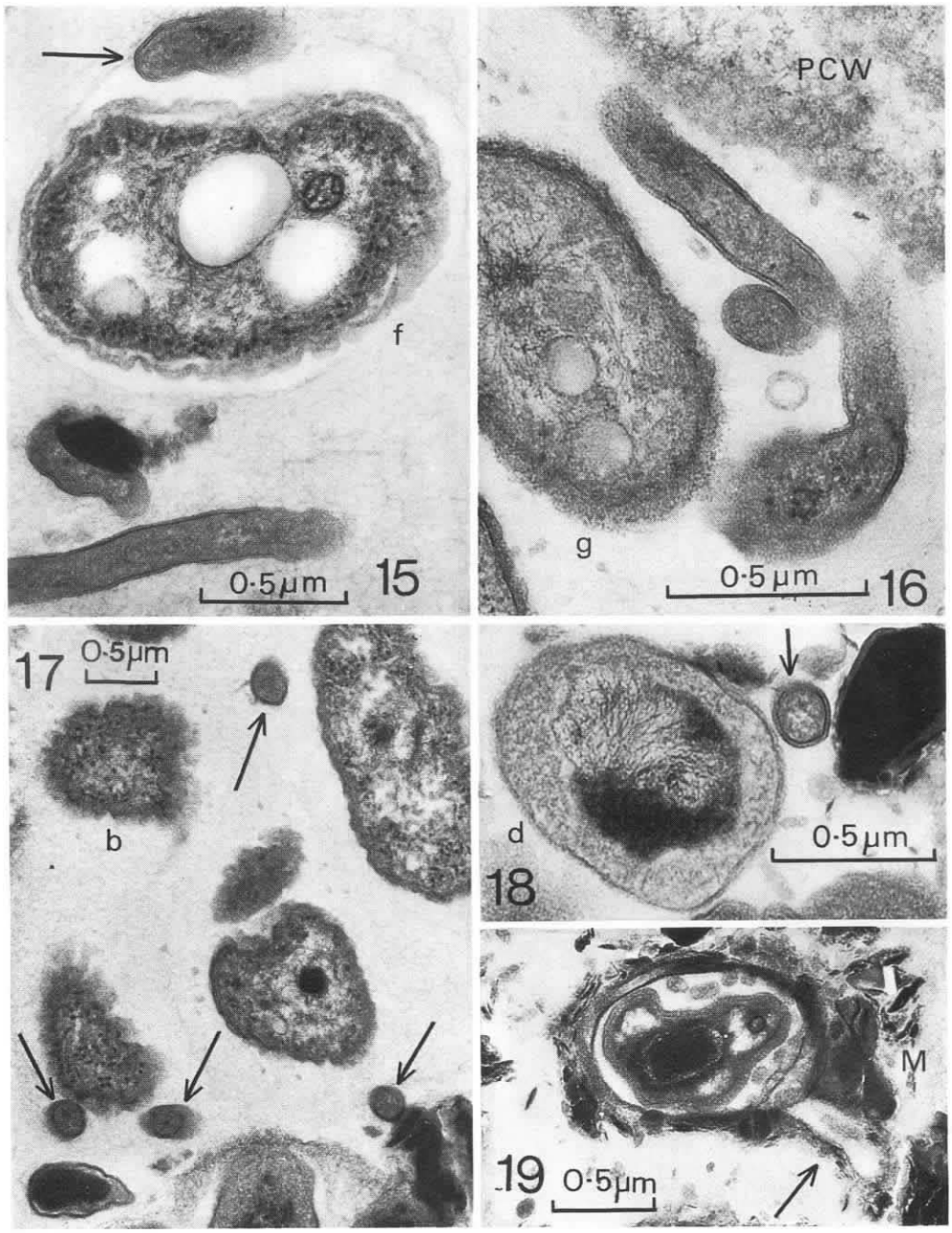
I. POSSIBLE PARASITIC RHIZOSPHERE ORGANISMS

Close examination of Figure 1 reveals that throughout the rhizosphere tubular organisms which are much smaller than the majority of bacteria occasionally occur (Figs. 15-19); they are circular in transverse section and are about 0.2 to 0.4 µm in diameter but their length cannot be determined from ultrathin sections. They have dense cytoplasm which shows some differentiation into a central fibrous nucleoid region (Fig. 15) and a peripheral granular region. They are bounded by a double membrane (Figs. 15 & 16) of the Gram negative type. They occurred close to several quite distinct types of organism as shown in Figures 15 to 18. It does not seem possible to us therefore that these tubular profiles can be parts of the larger bacteria, and we believe that they are independent soil organisms. Similar elongated forms have been illustrated in whole-mount, negatively-stained preparations (14) and in ultrathin sections (1) and identified as Bdellovibrio. Bdellovibrio are known to occur in Australian agricultural soils, and to attack Rhizobium and other soil bacteria (20).

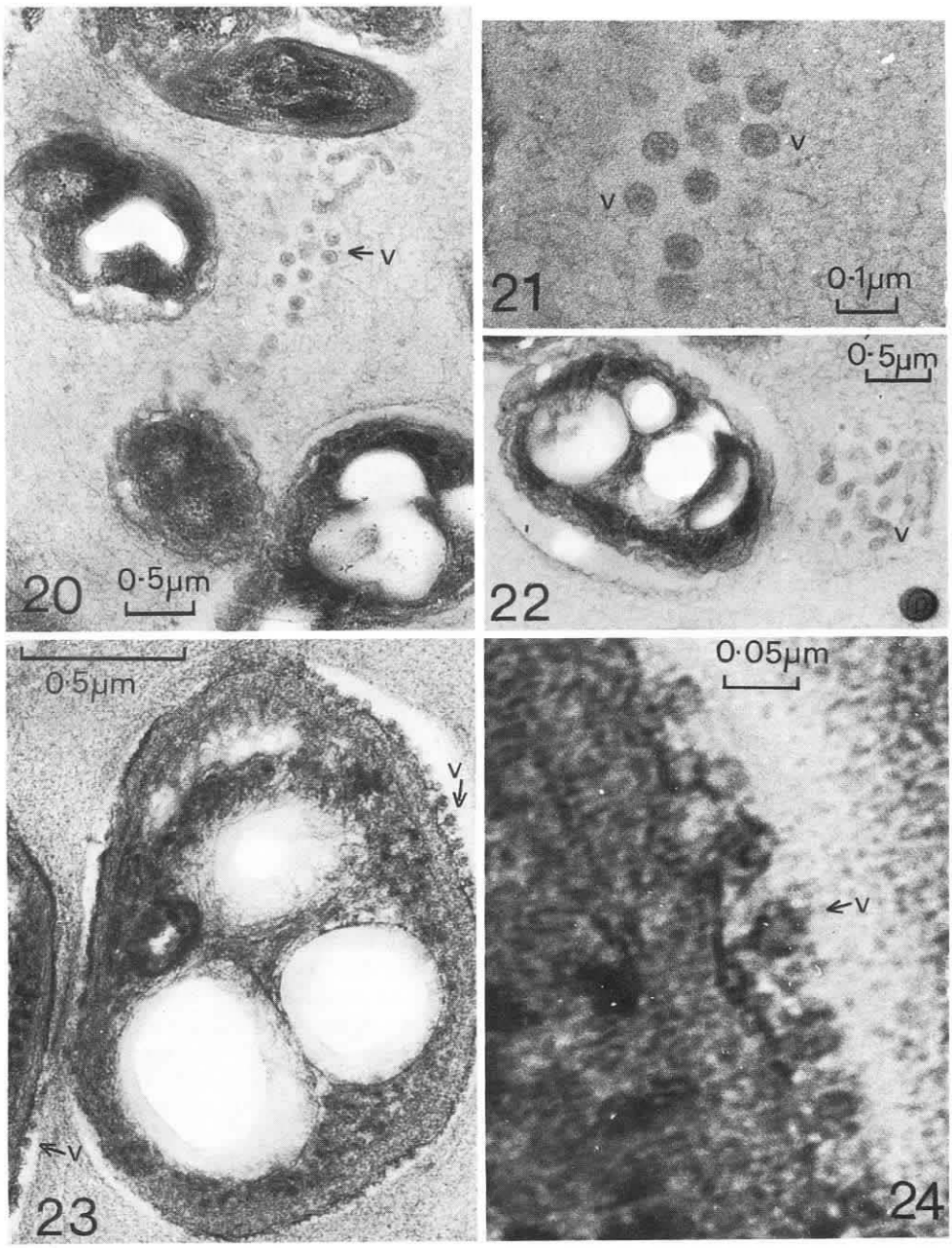
Also embedded in the rhizosphere matrix there are clusters of very small particles, usually associated with 'f'-type bacteria, (Figs. 20 & 22). The particles lie in an amorphous zone surrounded by matrix material (Figs. 20 & 21) and do not appear to have any particular orientation with respect to the adjacent bacterial



Figs. 11-14. Lobed rhizoplane organisms (see legend to Fig. 1)



Figs. 15-19 (see legend to Fig. 1)



Figs. 20-24. Virus-like particles (see legend to Fig. 1)

cells. We believe that these particles are bacteriophage as they are about 0.05 - 0.01 μm diameter, which is of the right order for bacteriophage (2) and they appear to be angular in cross section with an electron-dense core (Figs. 21-24).

D. Discussion

This study has shown that the whole of the rhizosphere of lateral roots of clover is permeated by a matrix of fine fibrils in which individual bacteria and colonies are embedded. The matrix extends to at least 20 μm from the root surface in some places, penetrating between the larger soil mineral grains and pushing the smaller clay particles to one side so that they form electron dense boundaries to the smaller bacterial colonies. Sometimes there is a distinct space between the matrix and the colonies of bacteria and between the matrix and individual bacteria suggesting that the matrix is different from the bacterial capsule materials.

It has been known for many years that roots secrete a wide range of organic materials into the rhizosphere (4, 17, 22). Barber and Martin (5) have recently shown that non-sterile roots grown in either simulated or natural soils secrete many times as much carbohydrate into the rhizosphere as sterile roots. Some of these secretions form a distinct mucilagenous layer on the surface of roots (6, 7, 16) and can be seen on roots grown under sterile conditions (7) as well as on roots grown in field soils (12, 13). In other TEM studies we have shown that the mucigel may be very extensive indeed forming a layer 10 or 20 μm thick over the surface of the root and it may have a fibrous appearance.

It is also well known however, that many species of bacteria produce extensive external deposits of fibrous carbohydrate both in pure culture and in mixed microbial associations in natural environments (9, 15). Bacteria obtained by centrifugation of soil suspensions sometimes have fibrous coats (3). Jones et al (15) showed that there is sometimes a transparent amorphous 'void' between the bacteria and the fibrous layer similar to that seen in Figures 6 and 15. These facts suggest that the fibrous matrix in the rhizosphere of the lateral roots of older clover consists mainly of capsule material produced by the bacterium type 'f' and not mucigel secreted by the host.

The density of microorganisms in the Trifolium rhizosphere ($5 \times 10^{10} cm^{-3}$) is about three orders of magnitude greater than that obtained by conventional plating techniques. Moreover the volume of soil sampled in Figure 1 contains at least eight different kinds of organism, since this number can be distinguished cytologically. This must mean that the root has contacted at least eight morphologically distinct founder microorganisms, which is many more than would be expected in so small a volume of soil, and it would appear that in order to account for the variety of organisms seen in Figure 1 there must have been migration of organisms from the surrounding soil into the rhizosphere.

In view of the richness of the rhizosphere in bacterial types and numbers it would be surprising if predatory organisms had not exploited this environment. Both Bdellovibrio and bacteriophage are usually obtained from soils and we believe that our interpretation of our electron micrographs is not inconsistent with the data presented in the literature (14) and that both these organisms are present in the clover rhizospheres. Close examination of wheat rhizospheres from the same soil shows that these contain Bdellovibrio-like organisms also,(12) and they may play an important role in the control of rhizosphere organisms. If our interpretation is correct, this is to our knowledge the first demonstration by direct observation of Bdellovibrio and bacteriophage in rhizospheres of plants grown under field conditions.

E. Acknowledgements

We are pleased to acknowledge the considerable skill and patience shown by Mrs P. Udompongsanon in the preparation of this material for electron microscopy and we thank Mr. T. Cock for preparing the plates for publication.

F. References

1. Abram, D. & B.K. Davis: Structural properties and features of Bdellovibrio bacteriovorus. J. Bact. 104: 948-965 (1970).
2. Ackerman, H.W.: The Morphology of Bacteriophages. In Laskin A.I. & H.A. Lechatilier, Handbook of Microbiology, Vol. 1. pp. 573-607 (1972). CRC Press, Cleveland, Ohio, USA.
3. Bae, H.C., E.H. Cota-Robles & L.E. Casida: Microflora of soil as viewed by transmission electron microscopy. Appl. Microbiol. 23: 637-641 (1972).
4. Barber, D.A. & K.B. Gunn: The effect of mechanical forces on the exudation or organic substances by the roots of cereal plants grown under sterile conditons. New Phytol. 73: 39-45 (1974).
5. Barber, D.A. & J.K. Martin: The release of organic substances by cereal roots into soil. New Phytol. 76: 69-80 (1976).
6. Campbell, R. & A.D. Rovira: The study of the rhizosphere by scanning electron microscopy. Soil Biol. Biochem. 6: 747-752 (1973).
7. Dart, P.J. & F.V. Mercer: The legume rhizosphere. Archiv. f. Mikrobiol. 47: 344-378 (1964).
8. Dawes, C.J. & F. Bowler: Light and electron microscope studies on the cell wall structure of root hairs of Raphinus sativus. L. Amer. J. Bot. 46: 561-565 (1959).
9. Fletcher, M. & G.D. Floodgate: An electron microscopic demonstration of acid polysaccharide involved in the adhesion of a marine bacterium to solid surfaces. J. Gen. Microbiol. 74: 325-334 (1973).
10. Foster, R.C. & G.C. Marks: Observations on the mycorrhizas of forest trees. The rhizosphere of Pinus radiata D. Don. Aust. J. Biol. Sci. 20: 915-926 (1967).
11. Foster, R.C. & A.D. Rovira: The rhizosphere of wheat roots studied by electron microsccpy. Bull. Ecol. Res. Comm. (Stockholm) 17: 93-102 (1973).
12. Foster, R.C. & A.D. Rovira: Ultrastructure of the wheat rhizosphere. New Phytol. 76: 343-352 (1976).
13. Greaves, M.P. & J.F. Darbyshire: The ultrastructure of the mucilaginous layer on plant roots. Soil Biol. Biochem. 4: 443-449 (1972).
14. Horowitz, A.T., M. Kessel & M. Shilo: Growth cycle of predaceous Bdellovibrios in host free extract system and some properties of the host extract. J. Bacteriol. 117: 270-282 (1974).
15. Jones, H.C., I.C. Roth & W.M. Saunders: Electron microscopy study of a slime layer. J. Bacteriol. 99: 316-325 (1969).
16. Jenny, H. & K. Grossenbacher: Root-soil boundary zones as seen in the electron microscope. Soil Sci. Soc. Amer. Proc. 27: 273-277 (1963).
17. Martin, J.K.: ^{14}C-labelled material leached from the rhizosphere of plants supplied continuously with $^{14}CO_2$. Soil Biol. Biochem. 7: 395-399 (1975).
18. Old, K.M. & T.H. Nicolson: Electron microscopical studies of the microflora of sand dune grasses. New Phytol. 74: 51-88 (1975).
19. Old, K.M., S. Hallam & J.H. Nicholson: Helicallylobed bacteria in plant roots. Soil Biol. Biochem. 7: 73-75 (1975).

20. Parker, C.A. & P.L. Grove: Bdellovibrio bacteriovorus parasitizing Rhizobium in Western Australia. J. Appl. Bacteriol. 33: 253-255 (1970).
21. Roper, M.M. & K.C. Marshall: Modification of the interaction between E. coli and bacteriophage in saline sediments. Microbial Ecol. 1: 1-13 (1974).
22. Rovira, A.D.: Plant root exudates. Bot. Rev. 35: 35-57 (1969).